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Sensitized Fluorescence for the Detection of Polycyclic Aromatic Hydrocarbons

Arthur D. Little, Inc, Cambridge, MA

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16. ABSTRACT The report describes a fluorescent spot test, devised for polycyclic aromatic hydrocarbons (PAH), based on the sensitization of the inherent fluorescence of such compounds. On filter paper, 10 pg of PAH in a spot of 0.25 cm diameter can generally be visualized when treated with naphthalene. In the case of benzo(a)pyrene, 1 pg has been detected. This method has been shown to be specific for PAH with minimum interference from other compounds. The method may be used to estimate the general level (factors of 10) of PAH in samples to aid in decisions for further more specific analyses.

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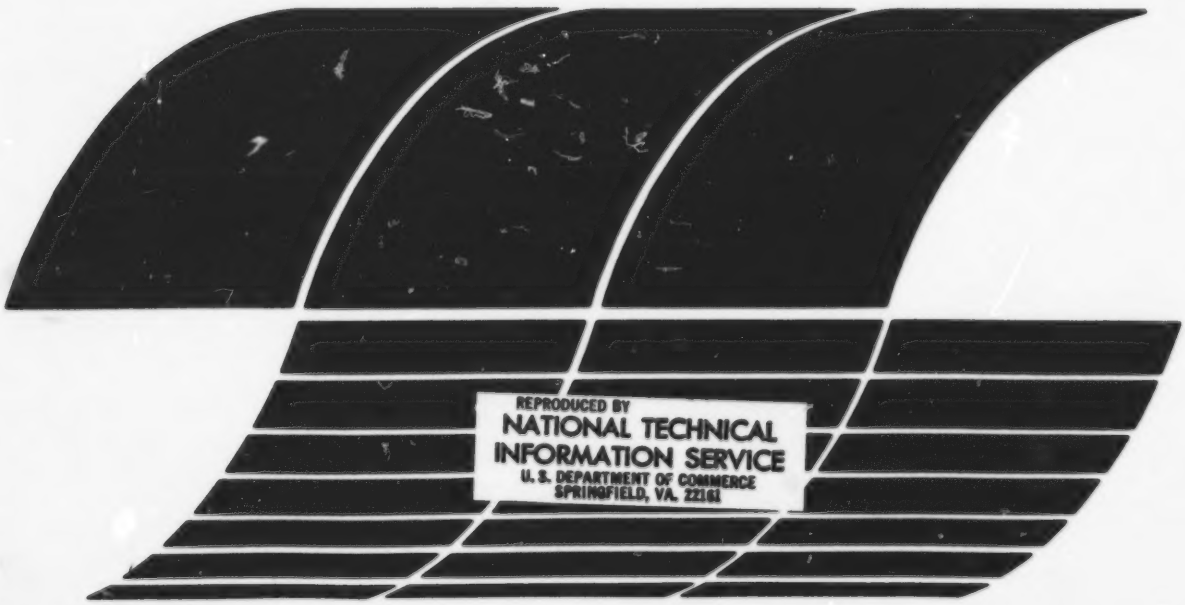

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Sensitized Fluorescence for the Detection of Polycyclic Aromatic Hydrocarbons

by

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Contract No. 68-02-2150
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EPA Project Officer: Larry D. Johnson

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I. SUMMARY

A fluorescent spot test has been devised for polycyclic aromatic hydrocarbons (PAH) based on the sensitization of the inherent fluorescence of such compounds. The basic procedure involves spotting a filter paper with a small amount of the sample solution, adding naphthalene, in solution, to the spot and visually observing the fluorescence under illumination with a simple ultraviolet light source. On filter paper 10 pg of PAH in a spot of 0.25 cm diameter can generally be visualized when treated with naphthalene. In the case of benzo[a]pyrene, 1 pg has been detected.

This method has been shown to be specific for PAH with minimum interference from other compounds. The method may be used to estimate the general level (factors of 10) of PAH in samples to aid in decisions for further more specific analyses.

II. INTRODUCTION

This project was initiated to determine whether the phenomenon of sensitized fluorescence could be utilized in the analysis of polynuclear aromatic hydrocarbons (PAH) as a class. A major objective was to develop a simple procedure for detection of PAH at much lower levels than current methods based on fluorescence analysis. This procedure, requiring only instrumentation readily available to most laboratories, would provide a low cost screening technique to determine whether environmental assessment samples contained levels of PAH such that more detailed analyses should be undertaken.

The PAH are inherently fluorescent materials and are known to exhibit sensitized fluorescence. The two processes of directly excited fluorescence and sensitized fluorescence are presented in a simplified energy level diagram in Figure 1.

Compound A, as depicted, absorbs energy (↑) and is raised to various excited singlet energy levels. An energy release is made vibrationally until the lowest excited singlet state is achieved (↓). The energy is released from this state to the various vibrational levels of the ground state in the form of fluorescent emissions (↓). When compound B is present, a vibrational coupling interaction can occur between the excited states of Compound A and Compound B[1], resulting in resonant energy transfer and fluorescent emissions of Compound B will be observed. As can be seen in the diagram, Compound B must have a common vibrational frequency with Compound A and its lowest vibrational level of the excited singlet state must be at lower energy than the corresponding level for Compound A. The transfer of energy is most efficient when the acceptor (B) is present in an extremely low molar ratio to the donor (A). Notable examples of sensitized fluorescence are naphthacene in benz[a]-anthracene[2] and in anthracene[3] at molar ratios of 10^{-4} and 10^{-6}, respectively.

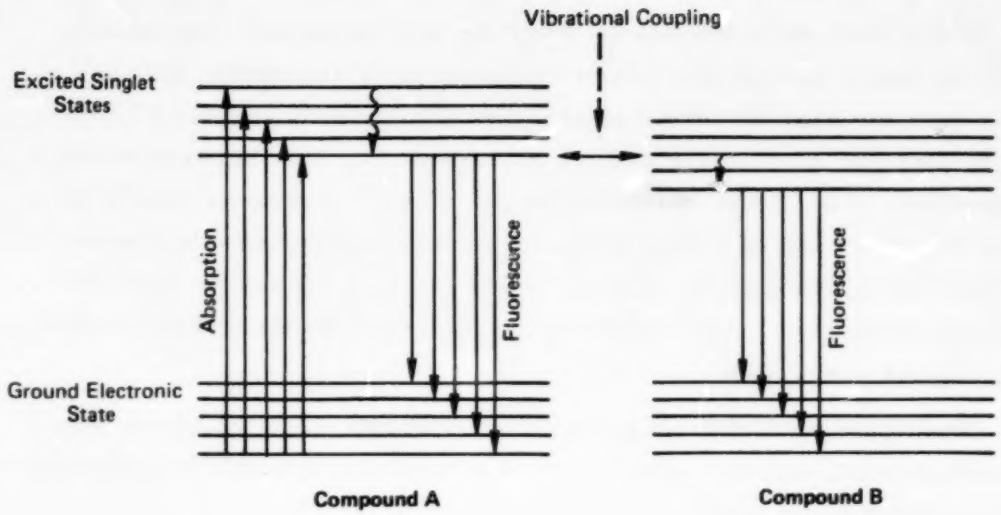


FIGURE 1 SINGLET – SINGLET ENERGY TRANSFER

In most studies involving directly excited fluorescence, the limit of detection of PAH has been on the order of 10 ng/mL in solution or, in the case of thin layer chromatography, 10 ng/spot. With sensitized fluorescence it was considered likely that by using the analyte as the "minor" constituent of an appropriate mixture, the limit of detection could be reduced on the order of 10^4 to 10^6-fold as noted in the above-mentioned sensitized fluorescence systems.

Among aromatic hydrocarbons, both the absorption and fluorescence shift to longer wavelengths (lower energies) with increasing conjugation; both are also at longer wavelengths for linearly conjugated compounds than for corresponding non-linear isomers. From the energy considerations, then, lower molecular weight aromatic compounds should be sensitizers for PAH of higher molecular weight (anthracene-naphthacene system) and in the case of isomers, a non-linearly conjugated aromatic compound should sensitize a linearly conjugated compound (benz[a]anthracene-naphthacene system).

Some lower molecular weight aromatic compunds might sensitize the fluorescence of many PAH and thus be of general use for PAH detection in a screening type of test.

III. DISCUSSION

1. Model Analytes

The PAH of interest in environmental assessment are those containing three or more fused rings. The known carcinogenic PAH contain at least four fused rings. In the benzenoid series, the low molecular weight analytes would be the isomers phenanthrene and anthracene of which only anthracene fluoresces in the visible. Higher molecular weight PAH, e.g., pyrene and chrysene, show fluorescent peaks in the ultraviolet but still have bands in the visible. PAH containing even more condensed rings all exhibit fluorescence in the visible region of the spectrum.

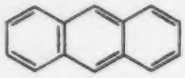
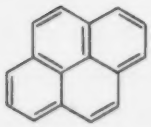
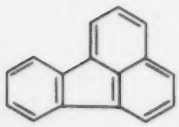
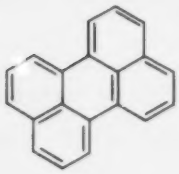
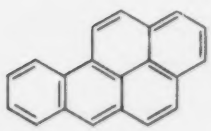
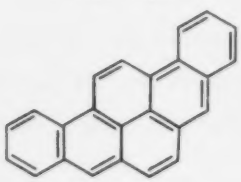
Similar spectral emission qualities are found in the methylene bridged PAH, e.g., fluorene derivatives. Fluorene and the benzofluorenes fluoresce in the ultraviolet and compounds with more fused rings emit in the visible region.

In order to meet the objective of keeping the method simple, only materials known to fluoresce in the visible spectral region were studied as analytes. In Table 1 are shown the compounds selected as representative analytes, their molecular structure, corrected fluorescent emission peaks[4] and carcinogenicity rating[5]. These compounds were chosen because of their range of molecular weights and their availability. (One factor that complicated this study is that many PAH recognized as carcinogens are no longer readily available.)

Although only non-heterocyclic compounds were tested as analytes in this study, the heterocyclic compounds bridged to aromatic ring structures are considered as analytes that can also be detected by sensitized fluorescence. The lower molecular weight members that fluoresce only in the ultraviolet region should be efficient sensitizers for the higher-molecular weight compounds, e.g., carbazole for the benzocarbazoles.

TABLE 1

MODEL ANALYTES FOR SENSITIZED FLUORESCENCE OF PAH

Compound	Structure	M.W.	Emission Peaks[4] nm	Carcinogenicity[5]
Anthracene		178	378, 400, 422	–
Pyrene		202	370, 382, 392	–
Fluoranthene		202	465	–
Perylene		252		–
Benz[a] pyrene		252	392, 416	+++
Coronene		300	425, 442, 450	–
Dibenzo[a,i] pyrene		302	420, 450	+++

–, Not Carcinogenic +++, Highly Carcinogenic

2. Fluorescence Sensitizers

In accord with the energy considerations mentioned earlier, lower molecular weight aromatic hydrocarbons were selected as potential fluorescence sensitizers. These included benzene, naphthalene, fluorene and phenanthrene, all of which absorb and fluoresce in the ultraviolet region of the spectrum. Their fluorescence emission bands are shown in Table 2. It should be noted that phenanthrene is indicated to have some fluorescence in the visible portion of the spectrum. As will be discussed later, a reasonable assumption is that a small amount of impurity present is responsible for the visible emission attributed to phenanthrene.

TABLE 2

Fluorescence of Sensitizers[6]

Compound	Emission Wavelength (nm)
Benzene	255 - 300
Naphthalene	300 - 365
Fluorene	302 - 370
Phenanthrene	348 - 407

In keeping with the objective for simplicity, observations of sensitized fluorescence were made with the unaided eye. Some comparisons of detection were made with a fluorescence spectrophotometer although the major use of such instrumentation was in checking the purity of analytes and sensitizers. Furthermore, since the sensitization is reported to be much more efficient in the solid state[1,2,3] than in solution, the study was directed toward simple procedures for producing small amounts of solid sensitizer/analyte mixtures.

Benzene, being a liquid at room temperature, was tested once in the solid state to confirm the presumption of its sensitizing effect. A solution of 10 pg perylene in 1μL benzene was frozen on a glass microscope slide. Excitation (254 cm) of the mixture resulted in the

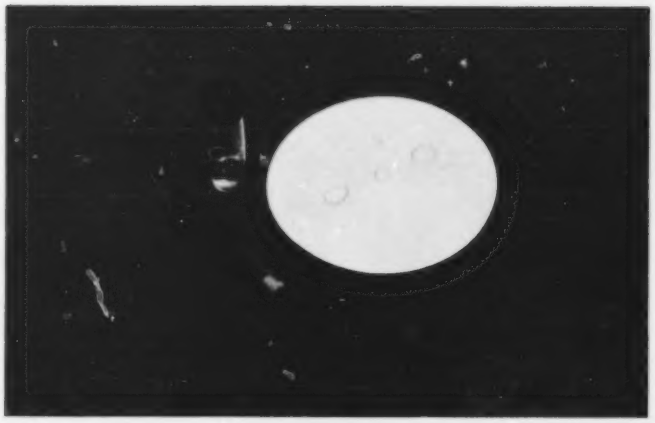
fluorescence of the perylene. Neither solid benzene (1μL ≈ 900μg) nor pyrene (100 pg) exhibited fluorescence alone.

Naphthalene was found to sensitize the fluorescence of all the model analytes, both in the form of crystalline mixtures on microscope slides and as mixtures in small spots on filter paper. The sensitization occurred with 10 pg amounts of the PAH and even 1 pg in the case of benz[a]pyrene (BaP). At that level, however, the fluorescence disappeared in about one minute, presumably due to loss of the BaP, either due to photoxidation or vaporization. At the 10 pg level the fluorescence of dibenzo[a,i]pyrene was very strong. The other analytes, however, were considered to be at about the minimum level of detectability. The naphthalene itself gave a weakly visible blue-white background that might be due to impurity or to scatter of the blue wavelengths that passed the filter of the 254 nm source. Because of the low intensity level of this background light, the exact nature of the background was not determined.

The naphthalene - PAH sensitized fluorescence detection was found to be readily accomplished in the form of a spot test on filter paper. A 1μL solution of the sample was applied to a small area (0.25 cm diameter) in the center of the paper and the same volume of sensitizer solution (60μg/μL) then added. Sensitizer and analyte background spots were applied (1μL each solution) to areas on either side of the mixture. As mentioned before, the naphthalene does have a visible background; at 10 pg and even 100 pg the fluorescence of model analytes alone was not evident.

The simplicity of the system is illustrated in Figure 2 showing the equipment and application of the test spots. Disposable capillary micropipets rated at 1μL are used for sample application. Pencil marks are drawn on the filter paper around the spot application areas. To minimize spot size, the sample spots are allowed to dry before application of the sensitizer spots. (The reverse order of application works just as well.) Figure 3 schematically indicates the appearance of the filter paper when illuminated at 254 nm.

a Filter paper
pipets
pipettor



b Pipetting
solution
by capillary
action

c Application
of solution
to test area

FIGURE 2 EQUIPMENT AND PROCEDURE

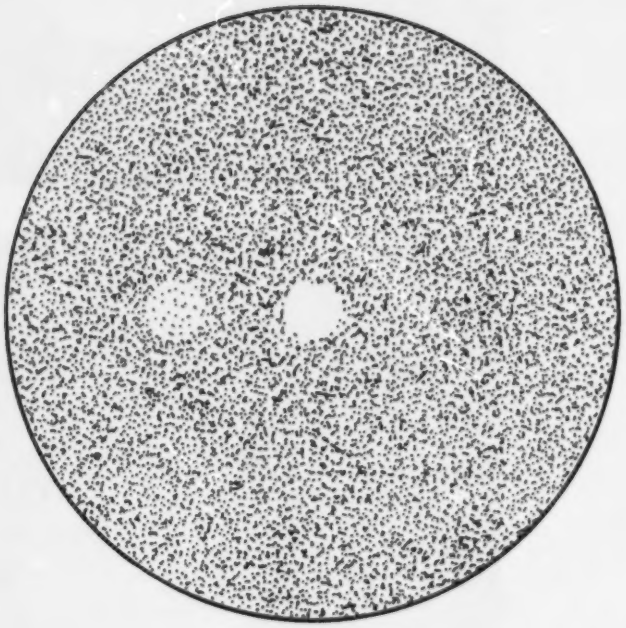


FIGURE 3 FLUORESCENT EMISSION OF NAPHTHALENE – PAH

The sensitivity of the system is even greater than originally expected in that the mole ratio of sensitizer to analyte is on the order of 10^{-7} in the case of 10 pg of four-ring PAH. At increased loadings of analyte, e.g., 100 pg, the intensity of fluorescence is indeed greater. The same effect is noted with lower amounts of sensitizer, e.g., 6μg naphthalene with 10 pg analyte. However, the volatility of naphthalene is such that with 6μg the fluorescing mixture disappeared in less than two minutes at room temperature. With 60μg of naphthalene, the fluorescence persisted on the order of 10-15 minutes.

Although naphthalene worked well as a sensitizer for all the model analytes, higher molecular weight aromatic compounds were considered as potentially capable of greater sensitivity and selectivity because of their closer approach to the PAH structures. Fluorene and phenanthrene appeared especially suitable because their inherent fluorescence was only in the ultraviolet spectral region and they did not sublime so readily as naphthalene at room temperature. However, studies in an attempt to use these sensitizers showed that all available samples of both fluorene and phenanthrene exhibited intense visible fluorescence in the solid state. The fluorescence, ascribed to PAH impurities in the materials, interfered in the detection of 10 pg amounts of the model PAH analytes.

Microscale purification of fluorene by paper chromatography indicated that it would sensitize the fluorescence of fluoranthene very well. Some efforts were undertaken to obtain pure fluorene and phenanthrene for use as sensitizers and the measures are described in a following section (III,5). The purification never reached the desired level of complete removal of fluorescent contaminants.

3. Instrumentation and Substrates

When it became evident that the sensitized fluorescence would allow visual observation of picogram quantities of PAH, emphasis was placed on developing a spot test technique that would require only simple instrumentation. For ultraviolet exposure of the samples, a

Chromatovue Cabinet, Model C-5, (Ultraviolet Products, Inc., San Gabriel, California) was found to be generally effective. This unit contains both 254 and 365 nm lamps of which the 254 nm source was used for the naphthalene - PAH fluorescent detections. The filter in this unit does, however, pass some wavelengths in the blue that are scattered by the sensitizer spot and substrate. Hand-held units such as the same firm's Model SL 2357 Mineralight do not have the necessary intensity and in addition transmit portions of the wavelengths in the red as well as the blue. These hand-held units can, however, be used with high (100 pg) amounts of PAH. From private communication with other users of 254 nm lamps it was determined that the 254 nm filters deteriorate with usage - indeed measurements of the output of the Chromatovue unit indicated only 200 $\mu W/cm^2$ three inches from the filter compared to claimed 760 $\mu W/cm^2$ eight inches away with a new unit.

Whatman #42 filter paper was selected as the most generally useful substrate after experimentation with quartz plates, glass slides, Eastman Type 301R2 Silica Gel chromatogram sheets, both Whatman #42 and Whatman #41 papers, Millipore Type GS filter and a transparent Teflon film. Some Whatman #42 lots have been noted to take on a green fluorescence on exposure to the 254 nm light, but that has not been found to interfere with the test for PAH.

4. Environmental Samples

Samples representative of those collected in environmental assessment studies were tested with the naphthalene reagent in order to explore the specificity and sensitivity of the method. Most of the samples had been previously analyzed for PAH by mass spectrometry or were so analyzed during the course of this study. The samples consisted of:

A. Effluents from thermal destruction of chemical wastes.
B. Particulates collected on filters from exhaust stacks.
C. Medicated shampoo (sold over-the-counter) labeled as containing 1% coal tar extract.

D. Fly-ash from power plant burning low-sulfur coal.

E. Mixtures prepared to test EPA Level 1 liquid chromatographic (LC) procedure.

F. 1,3,5-Trinitrotoluene (representing potential interferences).

A. Thermal Destruction Samples

A chemical waste thermal destruction study had been performed under a separate EPA contract and the following samples available from that study were examined.

a. Pentane extract of sorbent trap sample from API separator bottoms[7].

b. Combined knock-out trap, probe wash and filter sample from pyrolysis zone sample of rubber manufacturing waste[7].

c. Methylene chloride extract of ash from styrene tars[7].

d. Pentane extract of sorbent trap sample from fuel oil background run[8].

e. Methanol extract of sorbent trap sample from hot zone incineration of PVC waste[8].

f. Methylene chloride soluble portion from the dry impinger of the fuel oil background run[8].

g. Methylene chloride soluble fraction from probe wash and filter, incineration of PVC waste.[8]

Solutions of each of the above were prepared at 10 ng/μL and 100 pg/μL for testing of 1μL spots on Whatman #42 paper. At the higher level, all samples exhibited sensitized fluorescence. At 100 pg/spot the API oil waste sorbent trap sample did not show the sensitization; the other effluents did give the fluorescence but at lower intensities than did the 10 ng spots. The correlation with the low resolution mass spectral data is shown in Table 3.

TABLE 3

Comparison of LRMS Data and Sensitized Fluorescence Results on Chemical Waste Incineration Samples

Sample	MW ≥ 178 PAH by LRMS	Sensitized Fluorescence 10 ng spot	Sensitized Fluorescence 100 pg spot
a	low	moderate	0
b	moderate-high	high	moderate
c	moderate	high	weak
d	high	high	weak
e	(no data)	weak	--
f	none	0	--
g	(no data)	0	--

Another indication of the level of PAH present was the direct fluorescence of the solutions. Samples a through e fluoresced at 10 ng/μL and samples b and d still fluoresced at 100 pg/μL. All sensitized fluorescence was observed as blue-white although the non-sensitized spot of 10 ng of b gave a weak yellow fluorescence of itself.

B. Filter Samples

The particulates on filters (glass fiber) were from three processes:

a. Exhaust stack filter from EPA Method 5 sample train used in incineration of PVC waste[8].

b. Similar filter used in incineration of nitrochlorobenzene with #2 fuel oil[9].

c. Filters taken from the stack of a home-type oil burner using #2 fuel.

Filters a and b were very lightly colored (tan) and represent stack samples taken after water scrubbers.

Since only small portions of the filters from a and b above were available, extracts were made by wicking benzene or methylene chloride along a wedge-shaped piece of each lying on a microscope slide. In the case of sample a, no solid was found at the tip nor was there any evidence of color migration along the wedge. Naphthalene applied to the tip did not show any sensitized fluorescence. In the case of sample b, birefringent crystals formed on the slide just beyond the tip of the wedge as the solvent evaporated. These crystals were not fluorescent of themselves nor did they show any fluorescence when treated with naphthalene.

The filters of sample c were sufficiently large to process by Soxhlet extraction with methylene chloride and an ensuing level 1 LC separation. A blank filter was carried through the same procedure to allow an estimate of the amount of organic matter extracted from the particulates on the filter. The weights were obtained by difference between the combined weights of weighing cups, thimbles, and filters before and after extraction and were as follows:

	Filter 1	Filter 2	Blank Filter
Gross	5.7 mg.	4.1 mg.	3.8 mg.
Net	1.9 mg.	0.3 mg.	-

The three extracts were initially reduced in volume to 25 mL of which 1μL was tested with naphthalene. The filter samples did show positive sensitization and the blank showed none.

The extracts were next processed through Level 1 LC and fractions 2, 3, and 4 were brought to 10 mL volumes. Naphthalene produced strong blue-white fluorescence with 1μL spots each of LC fractions 3 and 4. GC/MS was run only on the number 3 fractions and confirmed the presence of PAH such as anthracene, chrysene and higher-molecular weight species.

C. Coal Tar Extract Shampoo

A medicated shampoo, labeled as containing 1% coal-tar, fluoresced intensely (yellow) under 254 or 365 nm radiation as did a hexane extract of 600 mg of the emulsion. The extract, reduced to 1 mL, was subjected to the Level 1 LC separation with fractions 2, 3, and 4 being collected and brought to 10 mL final volume each. Fraction 2 was clear and a 1μL portion showed no enhanced fluorescence with the naphthalene reagent. Fractions 3 and 4 were yellow colored and highly fluorescent. Fraction 3 was diluted 10^5-fold before a 1μL spot showed no visible fluorescence of itself. This spot did show sensitized fluorescence when treated with naphthalene although the result was estimated to be at the lower limit of detectability. LRMS of fractions 3 and 4 showed the presence of PAH ranging from fluorene (m/e 166) through the dibenzoperylenes (m/e 352).

This sample was used to evaluate the potential use of the fluorescent spot test in estimating concentration of PAH which might be detected in environmental samples. Certain assumptions were made, e.g., that the amount of PAH in fraction 3 was two times that in fraction 4 (an estimate based on the visible fluorescent intensity of the fractions). That estimate plus the assumption that the 1μL spot from the final dilution represented 1 to 10 pg of PAH led to the following calculation:

Fraction 3: $\frac{1 \text{ to } 10 \text{ pg}}{1\mu L} \times 10 \text{ mL} \times 1 \times 10^5 = 1 \text{ to } 10 \text{ mg}$

Fraction 4: 0.5 to 5 mg

Combined 1.5 to 15 mg

The original mass extracted was 600 mg and the estimated PAH content of the shampoo, then, would be in the range of 0.25 to 2.5% by weight.

D. Fly Ash Sample

A fly ash sample from a midwestern power plant was available for testing. This fly ash represented the residue from a low-sulfur coal. A small amount of the solid on a microscope slide was treated directly with the naphthalene sensitizer in benzene; no fluorescence was observed when the naphthalene crystallized.

E. Organic Level 1 LC Test Mixtures

Mixtures of organic chemicals were available which had been prepared for evaluating Level 1 LC procedures. The solutions were made up in pentane to contain 500 ppm of the compounds listed in Table 4.

TABLE 4

Level 1 LC Evaluation Mixtures

a	b	c
n-pentane	cumene	tetrachloroethane
n-octane	phenol	cumene
n-nonane	n-methylaniline	benzaldehyde
n-decane	acenaphthene	2-ethylhexanol
n-undecane	hexadecane	o-nitrotoluene
n-tridecane	4,4'-dichloro-biphenyl	quinoline
n-pentadecane		dihexylether
n-heptadecane		

The initial reason for considering these mixtures was to examine them for possible interferences in the sensitized fluorescence procedure. However, when it became evident that mixture b contained a fluorescent species, the evaluation changed to center on it. Mixtures a and c (1μL spots of them) did not show any fluorescence alone or in combination with naphthalene. A 1μL spot from mixture b did fluoresce and the fluorescence was enhanced with naphthalene. Portions of the mixture were spiked with anthracene, fluoranthene, perylene, pyrene, and coronene so that 10 pg/μL of the analytes would be present. Treatment with naphthalene of these PAH-spiked solutions of sample b gave rise to fluorescence of the same intensity as seen with the unspiked mixture.

Although the fluorescence could not be directly attributed to any of the components of the mixture because of the molecular structure, the possiblility of contamination was considered. Since acenaphthene and 4,4-dichlorobiphenyl are relatively low-molecular weight aromatic compounds, the original materials used to prepare the mixture were checked in solid form for fluorescence and noted to emit with yellow and blue-white colors respectively. Solutions of each were then prepared in methylene chloride approximating the concentration of each in the original mix, 300 ng/μL. At this concentration, a 1μL spot from the acenaphthene solution was non-fluorescent of itself but strongly fluorescent (yellow) where sensitized with naphthalene. The equivalent dichlorobiphenyl spot fluoresced alone and was strongly sensitized by naphthalene.

Each of the compounds (100-400 ng) was analyzed by GC/MS. Using capillary columns, both acenaphthene and 4,4-dichlorobiphenyl did not reveal any species that would account for the observed fluorescence. The acenaphthene was seen to contain a small amount of material of m/e 178 (anthracene and/or phenanthrene). No higher-molecular weight aromatic compounds were found in the dichlorobiphenyl. It was concluded that both components must have contained PAH as contaminants and in the solid state each acted itself as sensitizer for the fluorescence of the impurities. It was also concluded that the acenaphthene and dichlorobiphenyl did not interfere with the detection of the impurity by naphthalene.

F. Nitroaromatic Compounds

Nitroaromatic compounds are known to interfere in fluorescence analysis by a quenching mechanism[6]. Trinitrotoluene (TNT) was used as a model of such compounds to determine its effect on the naphthalene-sensitized fluorescence of anthracene. At high concentrations, 2μg TNT to 10 pg anthracene in 60μg naphthalene, the fluorescence of anthracene was indeed quenched. But at 1 ng TNT (still 10^2 times the anthracene concentration), the sensitized fluorescence of the anthracene was definitely observed. These compounds are therefore viewed as minor interferences.

5. Attempted Purification of Sensitizers

As indicated in Section III,2, low molecular weight PAH were also considered potential sensitizers for fluorescence, particularly phenanthrene and fluorene. GC/MS and fluorescence analysis of solid phenanthrene and fluorene showed that both contained anthracene as the principal visible fluorescent contaminant. Figure 4 demonstrates the effect of the emission spectra of the anthracene in phenanthrene. In solution (0.01 mg/mL benzene) the emission is that of phenanthrene with a shoulder at 400-405 nm attributed to the presence of a trace amount of anthracene. The emission curve obtained on the solid phenanthrene is really that of anthracene, a case of sensitized fluorescence.

High performance liquid chromatography (HPLC) was investigated as a method to purify fluorene and phenanthrene. Initially, size exclusion chromatography (SEC) on a μ-Styragel column was attempted, using tetrahydrofuran as solvent. Detection of components was with a differential refractometer. Solids obtained from different fractions collected during SEC were observed for fluorescence, or lack of it, under 254 and 365 nm radiation. The solids that were recovered always exhibited fluorescence, indicating a lack of adequate separation of undesired impurities from the bulk samples. A reverse phase column system, using a Partisil 10-ODS column, appeared to give good separation using small volume injections (10μL). However, attempts to use such a system for preparative work failed due to the small capacity of the column, the amount of sample involved, the loss of solids on evaporation, and eventual loss of the separating capability of the column due to overloading.

Partial success was achieved with a liquid-liquid partitioning process reported[10] for removing anthracene from phenanthrene. The phenanthrene was dissolved in cyclohexane and the solution then treated with concentrated sulfuric acid. Six sulfuric acid extractions reduced the ratio of anthracene to phenanthrene drastically but never sufficiently to render the mixture nonfluorescent. Continued extractions appeared to

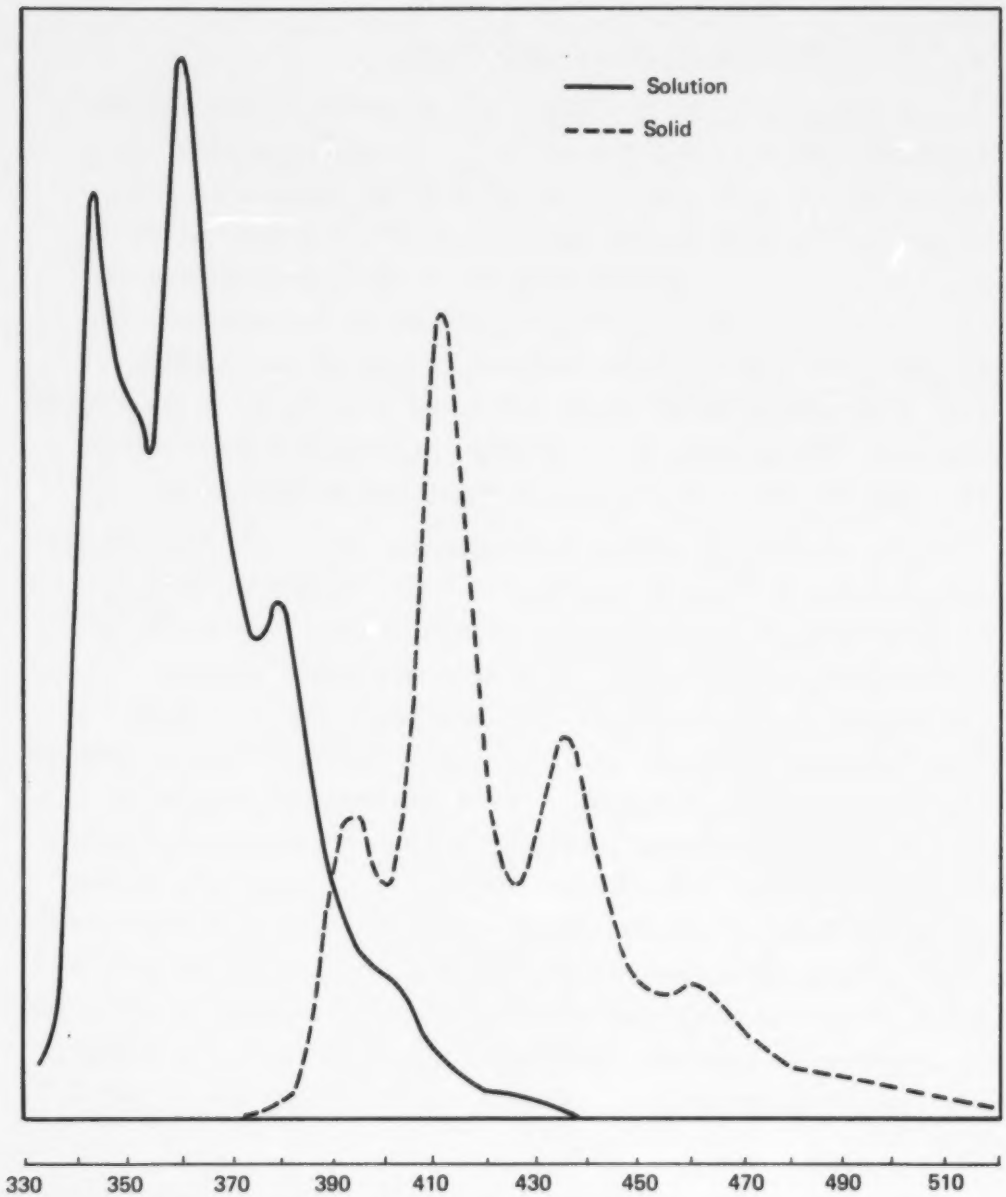


FIGURE 4 PHENANTHRENE FLUORESCENCE SPECTRA

result in chemical changes in the mixture. These results are presented in Figure 5 where the fluorescence emission spectrum for the starting material, the phenanthrene after six treatments, and the phenanthrene after 17 treatments are shown.

Fluorene was similarly partially purified by the partioning between sulfuric acid and cyclohexane. Results similar to those found with anthracene were observed.

The process with both compounds, however, was not adequately reproducible. Therefore, the sensitization of PAH fluorescence by naphthalene was made the method of choice.

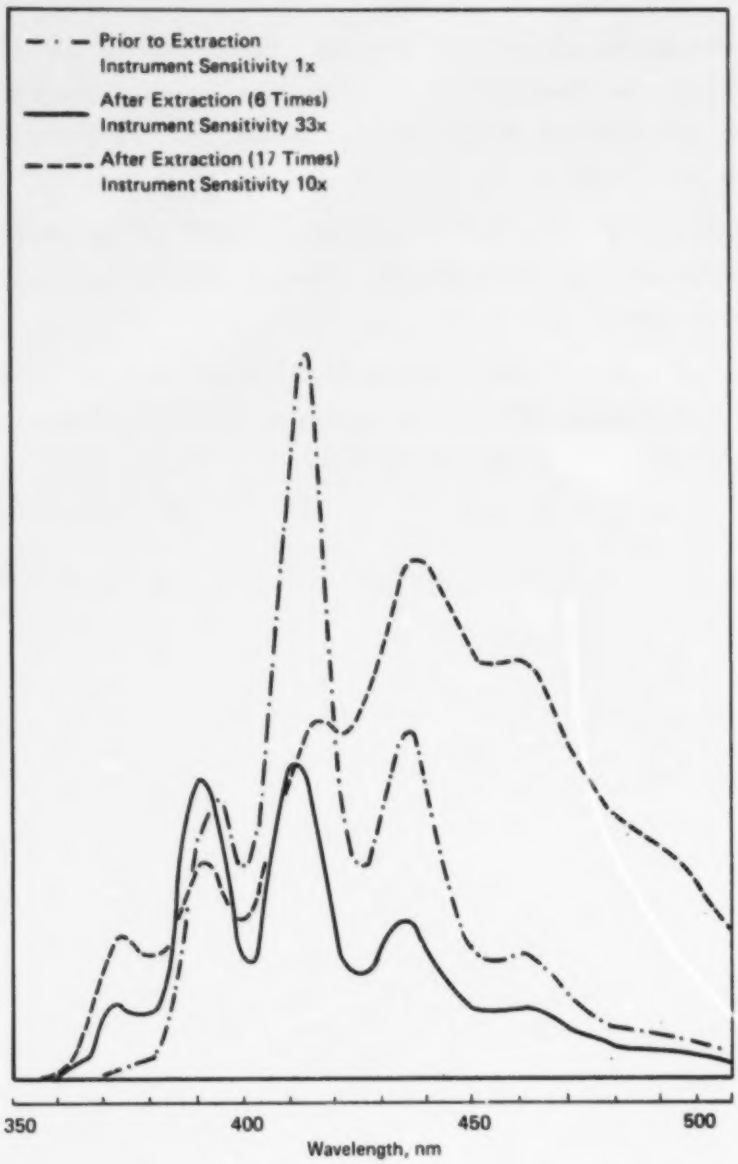


FIGURE 5 PHENANTHRENE EMISSION SPECTRA

IV. SUGGESTED APPLICATIONS

In its present form the sensitized fluorescence spot test is useful for screening environmental assessment samples for the presence of PAH at least as low as 10μg/L (pg/μL) in solution. The absence of sensitized fluorescence might well indicate that additional analyses for PAH are not necessary; on the other hand, a positive fluorescence test might indicate that GC/MS analyses should be performed to determine the exact nature of the PAH detected.

The positive identification of an individual PAH could be made based on the emission spectrum of the analyte-sensitizer mixture. The identification could be done with lower quantities of PAH than have been detected, e.g., with TLC procedures. The instrumentation would consist of a fixed wavelength excitation source and a monochromator for the emission spectra.

With the use of fluorescence spectrophotometers, the purity of the analyte might not be so critical since the fluorescence due to the trace contaminant would be known. The increase in intensity due to addition of PAH in an analyte could be measured readily.

V. SPOT TEST ANALYSIS PROCEDURE

1. Principle: The fluorescence of a polycyclic aromatic hydrocarbon is greatly enhanced when it is present in trace quantities (10^{-4} to 10^{-6} mole ratio) in a solid aromatic hydrocarbon of lower molecular weight, e.g., anthracene in naphthalene. In the case of isomers, the less linearly conjugated one is a sensitizer for the more linearly conjugated one(s), e.g., phenanthrene for anthracene.

2. Range and Sensitivity: Many PAH can be detected at 10 pg in the presence of 6 to 60 µg of naphthalene. Benz[a]pyrene has been detected at 1 pg.

3. Interferences: highly-nitrated aromatic compounds are known to quench fluorescence of PAH. At trace levels, however, that effect is probably less likely than the transfer of energy to PAH.

4. Precision and Accuracy: Concentrations of PAH can be estimated within a factor of 10 in the sensitized fluorescent spot test by 1:10 serial dilutions of the sample.

5. Apparatus: (Sources are those used during study and equivalent sources are acceptable)

   5.1 Ultraviolet source, 254 nm (Chromatovue Model C5)

   5.2 Filter paper (Whatman #42)

   5.3 Pipets (Drummond Microcaps, 1µL)

6. Reagents:

   6.1 Naphthalene (Fisher Scientific Catalog #N-134, "Certified" 60µg/µL.

   6.2 Benzene or methylene chloride (Fisher Scientific, Spectroanalyzed Grade).

7. Procedure: This sensitized fluorescence spot test presupposes that the sample has been obtained in an organic solvent either by direct solution, extraction, or a separation procedure such as liquid chromatography.

7.1 With pencil, mark three circles on filter paper each approximately 0.25 cm in diameter.

7.2 With the paper supported so that marked spots are not in contact with any other surface, apply 1μL of sample solution to central portion of each of two marked spots. Allow to air dry, keeping spots from contacting other surfaces.

7.3 Similarly apply 1μL of naphthalene reagent solution to remaining blank circle and to spot containing sample.

7.4 Observe spots under 254 nm, viewing either side of substrate. Note whether differences in intensity or color exist between sample-reagent spot and either spot alone. Any difference indicates sensitized fluorescence. (At 1 ng PAH the fluorescence of the sample spot itself should not be evident.)

Since the limits of detection are 1 to 10 pg PAH/spot for sensitized fluorescence and approximately 10 ng/spot for non-sensitized fluorescence, the results of the spot test procedure can be used to make the following estimates of PAH contents in the μL of sample.

a) non-fluorescent when treated with sensitizer : $\leq$ 1 pg
b) weakly fluorescent when treated with sensitizer : 1-10 pg
c) strongly fluorescent when treated with sensitizer but not fluorescent alone : $\geq$ 100 pg
d) fluorescent without sensitizer : $\geq$ 10 ng

From such estimates, the decision to proceed with further analyses can be made. In the case of strong sensitized fluorescence, or fluorescence without sensitization, a better estimate of concentration may be made by directly testing dilutions of the sample solution.

8. Calculations: In order to determine the PAH level in the sample solution, the observation should be made on successive 1:10 dilutions until the sensitized fluorescence is no longer observed. Under the conditions for the test--a 1μL sample volume and 10 pg as the lowest detectable amount of PAH--the concentration of PAH in the solution can be

calculated (within a factor of 10) as follows:

$$C = 1 \times 10^{(n-6)} \text{ g/L}$$

where n = number of 1:10 dilutions.

(The above formula is derived from the more explicit one:

$$C = \frac{10 \times 10^{-12} \text{g}}{1 \times 10^{-6} \text{ L}} \times 10^{n-1}).$$

For example, a solution sample that was diluted eight (8) times to reach the point of no recognizable sensitized fluorescence would contain $1 \times 10^{(8-6)} = 100$ g/L.

Since the sample solution used in this test may be an extract, LC fraction, aliquot of another solution, or derived in some other way from an original environmental assessment sample, the appropriate factors must then be applied to compute the PAH content of that original sample.

9. Stability: The naphthalene sensitizer solution, kept in a tightly-stoppered dark brown bottle, has been found to be stable over a one-year period.

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